REMARKS/ARGUMENTS

Claims 66-72 and 78-90 are pending. Claims 1-65 and 73-77 have been canceled. Claims 67, 68 and 81-83 have been amended. Support for the amendments is found throughout the specification and are depicted in the drawings. Reconsideration of the rejection is respectfully requested.

Claims 67, 69-73 and 84-90 were rejected under 35 USC 102(e), as being anticipated by Vind. Vind is cited to prepare a heteroduplex and adding a cell extract, which is alleged to contain the recited activities, followed by recovering variant homoduplexes. This rejection is respectfully traversed.

The present invention concerns the synthesis of new polynucleotide variants using a composition with defined components. Unlike Vind who uses a cell extract with many enzymes, including the entire DNA repair system, the present invention works well with only specific enzymes. Claim 67 was amended to recite that the strand cleavage activity is provided by a mismatch recognizing and mismatch directed endonuclease. The DNA repair system used by Vind does not appear to have and certainly does not use a single enzyme with this activity. The use of a single enzyme in place of a system of many enzymes, which perform a different function, is not obvious.

The bacterial DNA repair system for cleaving mismatches is a complex series of many reactions by several enzymes working together which typically cleave far from the actual mismatch without much specificity of the cleavage location. This is not a single enzyme recognizing and cleaving specifically at the mismatch as in the claimed invention. This becomes more significant when one uses a heteroduplex of two parent polynucleotides where two or more mismatches are located close together. Using the traditional DNA repair mechanism, such as Vind, hundreds to thousands of bases are removed and replaced. Vind does not produce a variant that reassorts between mismatches that are only a few or few dozen bases apart. By contrast, the present invention with a specific mismatch recognition and mismatch cleavage endonuclease can separately cleave and separately reassort between mismatches only a few bases apart. Recent data analysis from the experiments given in the examples indicates that in one situation, two mismatches were present in less than 10 base

pairs of the heteroduplex. A resulting variant of the claimed method produced a double reassortment with apparently both mismatches separately cleaved and resolved to different strands.

Furthermore, it should be noted that Vind uses a microbial extract whereas the enzyme exemplified in the specification CEL I is from celery and is believed to be involved with redistribution of nutrients, perhaps for seed production, and is not part of the traditional DNA repair system. Accordingly, this rejection should be withdrawn.

Claim 68 was rejected under 35 USC 103 as being unpatentable over Vind. In addition to the reasons given above, the examiner contends that the particular order or any order of adding ingredients is obvious. This rejection is respectfully traversed.

As explained in the previously response, thc order of addition matters. Furthermore, Vind adds all of their enzyme components at once as a single composition, namely an extract. Vind never has the enzyme components separate. Therefore, Vind cannot teach adding the components in any separate order. If one adds the extract multiple times, the same enzymes in the same proportion are being added each time. It is not possible to separately add the enzymes in Vind. Therefore, it is contrary to the specific teachings of Vind to add any enzyme component separately because use of a cell extract is taught. Accordingly, this rejection should be withdrawn.

Claims 75-77 and 80 were rejected under 35 USC 103(a) as being unpatentable over Vind in view of Arnold et al. Claims 75-77 have been canceled. As for claim 80, Arnold et al is cited to show E. coli DNA repair extracts contain Pol 1. The Examiner concludes it obvious to have this present. This rejection is respectfully traversed.

Vind is concerned with using extracts with thermostable mismatch repair proteins. See column 31, line 53-57. Since the Vind method involves heating to DNA denaturing and annealing temperatures, thermostable proteins are required. E. coli enzymes are readily heat denatured and generally not considered thermostable. Therefore, one would not be motivated to use E. coli Pol 1 in the Vind method.

Still further, even combining these two references, one would still not have the claimed invention for the reasons given above regarding Vind. Accordingly, the rejection should be withdrawn.

Claims 66, 74 and 81 and 82 were rejected under 35 USC 103(a) as unpatentable over Vind in view of Oleykowski et al. In addition to the teachings mentioned above, the examiner urges Oleykowski et al teaches CEL I and that CEL I is superior to T4 endonuclease VII for mutation detection assays. The examiner concludes it obvious to use CEL I in the mismatch repair method of Vind. This rejection is respectfully traversed.

In addition to the comments in the previous reply, Oleykowski et al do not compensate for the deficiencies in Vind. The present invention is not a mutation detection assay but rather a method for making a large number of sequence variants. Vind is also not a mutation detection assay. Furthermore, Vind does not utilize T4 endonuclease VII (or compositions containing it) and therefore the superiority of CEL I is irreverent. CEL I is not taught to be part of the DNA repair system. There is no reason for adding an enzyme such as CEL I to Vind because CEL I is not taught to be a part of any DNA repair system, much less that of Vind. Accordingly, this rejection should be withdrawn.

Claims 78, 79 and 83 were rejected under 35 USC 103(a) as being unpatentable over Vind in view of Birkenkamp et al. The examiner considers it obvious to use the Birkenkamp et al mismatch correction system (or at least certain components) in the repair system of Vind. This rejection is respectfully traversed.

Since Vind wishes to generate a library of new polynucleotides (note the title) and Birkenkamp et al wish to correct a mismatch to one of the two parent strands, the goals appear opposite and it is unclear why one would want to combine the two techniques. Furthermore, Vind requires thermostable enzymes because Vind subsequently denatures and reanneals their DNA. The Birkenkamp et al enzymes are not sufficiently thermostable and thus it is not obvious to add something expected to be inactivated and thus useless in the process. Still further, the Vind DNA repair system (cell extract) is complete by itself and needs no supplementation, especially with the Birkenkamp et al system used for a different

purpose and which would be denatured by heat during the Vind process. Accordingly, this rejection should be withdrawn.

As a separate argument pertaining to more than one rejection, the compositions used in some of the claimed methods involves enzymes from more than one species, e.g. celery, bacteria and phage. The prior art references using a cell extract for their enzymes would inherently have all of their enzymes from only one species. Therefore, there would be no motivation and no practical way to prepare a cell extract with enzymes from multiple species.

In view of the amendments and comments above, the rejections have been overcome. Reconsideration, withdrawal of the rejections and early indication of allowance are respectfully requested. If any issues remain, the examiner is encouraged to call the undersigned for prompt resolution.

If needed, applicants petition for an extension of time under the provisions of 37 CFR 1.136(a) for sufficient time to accept this response. The commissioner hereby is authorized to charge payment of any fees under 37 CFR § 1.17, which may become due in connection with the instant application or credit any overpayment to Deposit Account No.500933.

Respectfully submitted,

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